Section I (Amendments to the Claims)

Please amend claims 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, and 46-48, as set out in the following listing of the claims of the application.

Please cancel claims 2, 5, 8, 14, and 62 without prejudice.

1. (Currently amended) A nucleic acid construct encoding a fusion protein, wherein the construct comprises a coding sequence for a protein of interest and a coding sequence for a ~~protease~~ subtilisin prodomain protein, wherein the fusion protein comprises the protein of interest operatively linked to the ~~protease~~ subtilisin prodomain protein, ~~and~~ wherein the ~~protease~~ subtilisin prodomain protein ~~has binding with high affinity~~ binds to a subtilisin ~~protease~~ or a variant thereof with a Kd of 10 nM or less, and wherein the subtilisin variant retains the activity of subtilisin.

2. (Cancelled)

3. (Currently amended) The nucleic acid construct according to claim [[2]]1, wherein the ~~protease~~ subtilisin prodomain protein further comprises one or more amino acid substitutions that increase binding affinity for subtilisin or a variant thereof, as compared to the ~~protease~~ subtilisin prodomain protein with no substitutions.

4. (Currently amended) The nucleic acid construct according to claim 1, wherein the ~~protease~~ subtilisin prodomain protein comprises a variant of SEQ ID NO: 1, wherein the variant comprises a substitution at one or more of positions P1-P4 wherein the substitution comprises any of F or Y substituted for P4, any amino acid residue substituted for P3, A or S substituted for P2 and M, F, Y H, or L substituted for P1.

5. (Cancelled)

6. (Currently amended) The nucleic acid construct according to claim [[5]]1, wherein the ~~protease~~ subtilisin prodomain protein comprises substitutions of amino acid residues F or Y for P4, any amino acid residue for P3, A or S for P2 and M, F, Y, H, or L for P1 at the C-terminal end.

7. (Currently amended) A fusion protein comprising a target protein operatively linked to a ~~protease~~ subtilisin prodomain protein, wherein the ~~protease~~ subtilisin prodomain protein is

modified to exhibit an increased affinity for subtilisin or a variant thereof, as compared to the unmodified ~~protease~~ subtilisin prodomain protein, and wherein the subtilisin variant retains the activity of subtilisin.

8. (Cancelled)

9. (Currently amended) The fusion protein according to claim [[8]]7, wherein the ~~protease~~ subtilisin prodomain protein comprises substitution of amino acids P4-P1with the amino acid sequence FKAM.

10. (Currently amended) The fusion protein according to claim 7, wherein the ~~protease~~ subtilisin prodomain protein comprises the amino acid sequence E E D K L (F/Y) Q S (M/L/Y) (SEQ ID NO: 7).

11. (Previously Presented) The fusion protein according to claim 7, wherein the target protein is staphylococcal Protein AB domain; Protein AB mutant A219; Streptococcal protein GB domain; Streptococcal protein Ga domain; Protein GB mutant G311; E. coli hypothetical Yab; Bovine a-subunit of transducin; M. thermautotrophicus CDC6; streptavidin; avidin; Taq polymerase; alkaline phosphatase; RNase; DNase; restriction enzymes; peroxidases; endo-1, 4-beta glucanase; endo-1, 3-beta-glucanase; chitinases; beta and alpha glucosidases; beta and alpha glucoronidases; amylase; glucosyl-transferases; phospho-transferases; chloramphenicol-acetyl-transferase; beta-lactamase; luciferase; esterases; lipases; proteases; bacteriocines; antibiotics; enzyme inhibitors; growth factors; hormones; receptors; membrane proteins; nuclear proteins; transcriptional factors; translational factors or nucleic acid modifying enzymes.

12. (Currently amended) A DNA construct for the preparation of a fusion protein, wherein the construct comprises a coding sequence of a protein of interest and a DNA sequence encoding a subtilisin binding protein ~~having binding with high affinity for~~ which binds to subtilisin with a Kd of 10 nM or less.

13. (Currently amended) A method for the production of a subtilisin binding fusion protein, the method comprising: providing a nucleic acid construct encoding a fusion protein wherein the fusion protein comprises a ~~protease~~ subtilisin prodomain protein operatively linked to a second protein of interest, wherein the ~~protease~~ subtilisin prodomain protein is modified to bind subtilisin or a variant thereof with increased affinity as compared to an unmodified ~~protease~~

subtilisin prodomain protein, and wherein the subtilisin variant retains the activity of subtilisin; transfecting a host cell with the nucleic acid construct; and culturing the transformed host cell under conditions suitable for expression of the fusion protein.

14. (Cancelled)

15. (Currently amended) The method according to claim [[14]]13, wherein the subtilisin prodomain is modified by replacing the P4 through P1 amino acids with FKAM, FKAY or FKAF.

16. (Previously Presented) The method according to claim 15, wherein the second protein of interest is staphylococcal Protein AB domain; Protein AB mutant A219; Streptococcal protein GB domain; Streptococcal protein Ga domain; Protein GB mutant G311; E. coli hypothetical Yab; Bovine a-subunit of transducin; M. thermautotrophicus CDC6; streptavidin; avidin; Taq polymerase; alkaline phosphatase; RNase; DNase; restriction enzymes; peroxidases; endo-1, 4-beta glucanase; endo-1, 3-beta-glucanase; chitinases; beta and alpha glucosidases; beta and alpha glucoronidases; amylase; glucosyl-transferases; phospho-transferases; chloramphenicol-acetyl-transferase; beta-lactamase; luciferase; esterases; lipases; proteases; bacteriocines; antibiotics; enzyme inhibitors; growth factors; hormones; receptors; membrane proteins; nuclear proteins; transcriptional factors; translational factors or nucleic acid modifying enzymes.

17. (Original) The method according to claim 13, wherein the host cells includes cells from, Escherichia coli, Bacillus, Salmonella, Pseudomonas; Saccharomyces cerevisiae, Pichia pastoris, Kluveromyces, Candida, Schizosaccharomyces; or CHO cells.

18. (Withdrawn) A method for purifying a protein of interest from a fusion protein and separation therefrom, the method comprising: contacting a fusion protein comprising a protease prodomain protein operatively linked to the protein of interest with an effective amount of subtilisin or a variant thereof under conditions suitable for the formation of a binding complex between the subtilisin or variant thereof and the protease prodomain protein of the fusion protein; incubating the binding complex for a sufficient time for the subtilisin or variant thereof to cleave the protein of interest from the binding complex; and recovering the protein of interest.

19. (Withdrawn) The method according to claim 18, wherein the subtilisin has been modified to specifically bind to the protease prodomain fusion protein.

20. (Withdrawn) The method according to claim 19, wherein the subtilisin includes mutations Q2K, S3C, P5S, K43N, A73L, deletion of 75-83, E156S, G166S, G169A, S188P, Q206C, N212G, K217L, N218S, T254A, Q271E, Y104A, G128S and at least one additional mutation at amino acid position 32, 155 or 221.

21. (Withdrawn) The method according to claim 19, wherein the protease prodomain protein is a subtilisin prodomain and modified by replacing the P4 through P1 amino acids with FKAM, FKAY or FKAF

22. (Withdrawn) The method according to claim 21, wherein the protein of interest is staphylococcal Protein AB domain; Protein AB mutant A219; Streptococcal protein GB domain; Streptococcal protein Ga domain; Protein GB mutant G311; E. coli hypothetical Yab; Bovine a-subunit of transducin; M. thermautotrophicus CDC6; streptavidin; avidin; Taq polymerase; alkaline phosphatase; RNase; DNase; restriction enzymes; peroxidases; endo-1,4-beta glucanase; endo-1, 3-beta-glucanase; chitinases; beta and alpha glucosidases; beta and alpha glucoronidases; amylase; glucosyl-transferases; phospho-transferases; chloramphenicol-acetyl-transferase; beta-lactamase; luciferase; esterases; lipases; proteases; bacteriocines; antibiotics; enzyme inhibitors; growth factors; hormones; receptors; membrane proteins; nuclear proteins; transcriptional factors; translational factors or nucleic acid modifying enzymes.

23. (Withdrawn) The method according to claim 20, wherein the subtilisin is immobilized on a solid phase matrix.

24. (Withdrawn) The method according to claim 21, wherein the prodomain of subtilisin is mutated to increase binding affinity of subtilisin to greater than 109 M^{-1}.

25. (Withdrawn) The method according to claim 19, wherein the subtilisin includes mutations Q2K, S3C, P5S, K43N, A73L, deletion of 75-83, E156S, G169A, S188P, Q206C, N212G, K217L, N218S, T254A, Q271E, Y104A, G128S and at least one additional mutation at amino acid position 32, or 221.

26. (Withdrawn) The method according to claim 20, wherein the subtilisin is S189, S190, S194, S196, S197, or S198.

27. (Withdrawn) The method according to claim 25, wherein the subtilisin is S199, S201 or S202.

28. (Withdrawn) An assay method for detecting the presence of a substance of interest in a test sample comprising: (a) incubating a test sample, which may contain a substance of interest, with a sufficient amount of a protease prodomain fusion protein, wherein the protease prodomain fusion protein comprises: (i) a protease prodomain capable of binding with high affinity to a subtilisin or variant thereof, and (ii) a second protein capable of binding the substance of interest, under incubating conditions that allow for the binding of the substance of interest to the second protein; (b) contacting the protease prodomain fusion protein used in step (a) to subtilisin or a variant thereof, wherein the subtilisin or a variant thereof is in solution in an amount effective to bind the fusion protein or immobilized on a solid phase to form a subtilisin/prodomain fusion protein binding complex; (c) incubating the subtilisin/prodomain fusion protein binding complex for a sufficient time for the subtilisin or variant thereof to cleave the second protein from the binding complex; (d) recovering the second protein bound to the substance of interest.

29. (Withdrawn) The method according to claim 28, further comprising introducing a detectable label capable of binding to the substance of interest; and determining the presence or absence of the label, to provide an indication of the presence or absence of the substance of interest in the test sample.

30. (Withdrawn) The method according to claim 29, wherein the detectable label is introduced before separation of the second protein from the binding complex or after the second protein is recovered.

31. (Withdrawn) The method according to claim 28, wherein the test sample is blood, urine, semen, saliva, mucus, tears, or vaginal secretions.

32. (Withdrawn) The method according to claim 31, wherein the substance of interest is an antibody.

33. (Withdrawn) The method according to claim 32, wherein the second protein is an antigenic receptor having affinity for the antibody.

34. (Withdrawn) The method according to claim 31, wherein the substance of interest is an antigen.

35. (Withdrawn) The method according to claim 34, wherein the second protein is an antibody having affinity for the antibody.

36. (Withdrawn) The method according to claim 28, wherein the subtilisin has been modified to specifically bind to the protease prodomain fusion protein.

37. (Withdrawn) The method according to claim 36, wherein the subtilisin includes mutations Q2K, S3C, P5S, K43N, A73L, deletion of 75-83, E156S, G166S, G169A, S188P, Q206C, N212G, K217L, N218S, T254A, Q271E, Y104A, G128S and at least one additional mutation at amino acid position 32, 155 or 221.

38. (Withdrawn) The method according to claim 28, wherein the protease prodomain protein is a subtilisin prodomain and modified by replacing the P4 through P1 amino acids with FKAM, FKAY or FKAF.

39. (Withdrawn) A drug delivery system comprising a subtilisin prodomain protein associated with a drug of interest to form a fusion product, wherein the fusion product is further complexed to a subtilisin or variant thereof to form a drug delivery complex.

40. (Withdrawn) The drug delivery system according to claim 39, wherein the drug of interest is conjugated to the subtilisin prodomain protein either directly or through a linker moiety.

41. (Withdrawn) The drug delivery system according to claim 39, wherein the drug of interest is slowly released from the drug delivery complex.

42. (Withdrawn) The drug delivery system according to claim 41, wherein the drug delivery product is included in a composition and administered parenterally, orally, topically or by inhalation.

43. (Withdrawn) The drug delivery system according to claim 41, wherein the composition comprises a solid, gel, liquid or aerosol.

44. (Withdrawn) The drug delivery system according to claim 41, wherein the subtilisin includes mutations Q2K, S3C, P5S, K43N, A73L, deletion of 75-83, E156S, G166S, G169A, S188P, Q206C, N212G, K217L, N218S, T254A, Q271E, Y104A, G128S and at least one additional mutation at amino acid position 32, 155 or 221.

45. (Withdrawn) The drug delivery system according to claim 41, wherein the subtilisin prodomain protein is modified by replacing the P4 through P1 amino acid residues with FKAM, FKAY or FKAF.

46. (Currently amended) A nucleic acid construct encoding a fusion protein, wherein the construct comprises a coding sequence for a protein of interest and a coding sequence for a second protein, wherein the second protein binds ~~with high affinity~~ to a subtilisin ~~protease~~ or a variant thereof with a Kd of 10 nM or less, and wherein the subtilisin variant retains the activity of subtilisin.

47. (Currently amended) A nucleic acid construct according to claim 46, wherein the fusion protein comprises the protein of interest linked to the second protein by a peptide bond and wherein the ~~protease~~ subtilisin hydrolyzes the peptide bond.

48. (Currently amended) A nucleic acid construct according to claim 46, wherein P1, P2 and P4 amino acids of the second protein generate affinity for S1, S2 and S4 binding pockets of the subtilisin ~~protease~~ or a variant thereof.

49. (Previously Presented) A nucleic acid construct according to claim 48, wherein the second protein comprises amino acid residues F or Y at the P4 position, any amino acid residue at the P3 position, A, S, V, or T at the P2 position and M, F, Y, H, or L at the P1 position.

50. (Withdrawn) A protease variant that is altered to specifically hydrolyze a fusion protein upon addition of a chemical trigger and the fusion protein comprises a binding sequence for a protease fused to a protein of interest.

51. (Withdrawn) A protease variant according to claim 50, wherein the altered protease is a subtilisin variant.

52. (Withdrawn) A protease variant according to claim 51, wherein the subtilisin variant comprises a mutation at amino acid 32.

53. (Withdrawn) A method of producing a protein of interest, comprising generating a fusion protein comprising a binding sequence for a protease fused to a protein of interest, and reacting said fusion protein with a protease variant that is altered to specifically hydrolyze the fusion protein and yield said protein of interest upon addition of a chemical trigger, wherein the reaction is conducted in the presence of said chemical trigger, and recovering said protein of interest.

54. (Withdrawn) A nucleic acid construct encoding a fusion protein, wherein the construct comprises a coding sequence for a protein of interest that is operatively linked to coding sequence for a peptide, wherein the peptide generates affinity for a protease or a variant thereof.

55. (Withdrawn) A nucleic acid construct according to claim 54, wherein the protease hydrolyzes a peptide bond joining the peptide to the protein of interest.

56. (Withdrawn) A nucleic acid construct according to claim 54, wherein P1, P2 and P4 amino acids of the peptide generate affinity for S1, S2 and S4 binding pockets of protease or a variant thereof.

57. (Withdrawn) A nucleic acid construct according to claim 56, wherein the peptide comprises amino acid residues F or Y at the P4 position, any amino acid residue at the P3 position, A, S, V, or T at the P2 position and M, F, Y, H, or L at the P1 position.

58. (Withdrawn) A method for the production of a subtilisin binding fusion protein, the method comprising: providing a nucleic acid construct encoding a fusion protein wherein the fusion protein comprises a peptide and a second protein of interest, wherein the peptide is modified to bind subtilisin or a variant thereof with high affinity; transfecting a host cell with the nucleic acid construct; and culturing the transformed host cell under conditions suitable for expression of the fusion protein.

59. (Withdrawn) A method for purifying a protein of interest from a fusion protein and separation therefrom, the method comprising: contacting a fusion protein comprising a peptide operatively linked to the protein of interest with an effective amount of subtilisin or a variant thereof under conditions suitable for the formation of a binding complex between the subtilisin

or variant thereof and the peptide of the fusion protein; incubating the binding complex for a sufficient time for the subtilisin or variant thereof to cleave the protein of interest from the binding complex; and recovering the protein of interest.

60. (Withdrawn) An assay method for detecting the presence of a substance of interest in a test sample comprising: (a) incubating a test sample, which may contain a substance of interest, with a sufficient amount of a fusion protein comprising: (i) a peptide capable of binding with high affinity to a subtilisin or variant thereof, and (ii) a second protein capable of binding the substance of interest, under incubating conditions that allow for the binding of the substance of interest to the second protein; (b) contacting the fusion protein used in step (a) to subtilisin or a variant thereof, wherein the subtilisin or a variant thereof is in solution in an amount effective to bind the fusion protein or immobilized on a solid phase to form a subtilisin/fusion protein binding complex; (c) incubating the subtilisin/fusion protein binding complex for a sufficient time for the subtilisin or variant thereof to cleave the second protein from the binding complex; (d) recovering the second protein bound to the substance of interest.

61. (Withdrawn) A drug delivery system comprising a peptide generating affinity for a subtilisin or a variant thereof associated with a drug of interest to form a fusion product, wherein the fusion product is further complexed to said subtilisin or variant thereof to form a drug delivery complex.

62. (Cancelled)